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### **Re: A Study of the Impact of Adding HPV Types to Cervical Cancer Screening and Triage Tests**

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In a recent article in the Journal, Schiffman et al. (1) showed that testing for more than about 10 human papillomavirus (HPV) types decreased specificity for detection of cervical intraepithelial neoplasia grade 3 (CIN3) and cancer more than it increased sensitivity in the ASCUS/LSIL Triage Study (ALTS) and, most notably, in the Proyecto Epidemiológico Guanacaste (PEG). To further elucidate which HPV types are the strongest predictors of the risk of CIN3 and cancer, we compared our three large systematic reviews on the distribution of HPV types in low-grade squamous intraepithelial lesions [LSILs; 8308 women from 50 studies (2)], high-grade squamous intraepithelial lesions [HSILs; 4338 women from 52 studies (3)], and squamous cell cervical carcinoma [SCC, 10 058 women from 85 studies (4)].

We compared the type-specific prevalences reported from our three systematic reviews, selecting the same HPV types highlighted by Schiffman et al.

(1), namely 13 high-risk HPV types (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) that are currently included in the DNA test approved by the Food and Drug Administration (FDA) as an adjunct to primary cytologic screening and for triage of women with equivocal cytology (5), as well as five additional types (i.e., HPV26, 53, 66, 73, and 82) that have been considered for inclusion in the DNA test (6). We found that HPV16 was twice as prevalent in HSILs as it was in LSILs. Other HPV types showed either a similar prevalence in LSILs and HSILs or a substantially higher prevalence in LSILs than in HSILs (Fig. 1).

Most important, the comparison of our systematic reviews provides additional insight into the shift in HPV type distribution in SCC, which was found in only 39 women in ALTS and PEG (1). In the International Agency for Research on Cancer (IARC) reviews, only HPV16 and 18 were found more frequently in SCC than in LSILs, whereas HPV26, 39, 51, 56, and 73 were at least 10-fold more common, and HPV53 and 66 were approximately 30-fold more common in LSILs than in SCC. The low-risk HPV types HPV6, 11, and 70 were also approximately 10-fold more common in LSILs than in SCC (data not shown).

Comparisons of HPV distribution in cross-sectional studies, as herein reported, have several limitations, including inaccuracies in cytologic/histologic classification and viral detection, as

well as non-negligible heterogeneity in HPV type distribution across different populations. HPV35 (7), for instance, was recently reported to be relatively common in HSILs and SCC in Mozambique, but little information on HPV type distribution in SCC in sub-Saharan Africa was available at the time of our systematic review (4). Nevertheless, the picture that emerges from our analysis of the IARC systematic reviews is remarkably consistent with the findings of receiver operating characteristic curves in PEG and ALTS. This picture suggests that 1) HPV types not currently included in FDA-approved DNA tests have little to contribute to SCC prevention, although this may also be the case for some of the types that are currently included (e.g., HPV39, 51, and 56); 2) HPV16 and 18, which are substantially enriched in SCC compared with LSILs and those types that are found at approximately equal frequencies in SCC and LSILs (HPV33 and 45) or slightly under-represented in SCC compared with LSIL (HPV31, 52, and 58) probably have the best trade-offs between sensitivity and specificity for cervical cancer screening prevention.

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## REFERENCES

- (1) Schiffman M, Khan MJ, Solomon D, Herrero R, Wacholder S, Hildesheim A, et al. A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J Natl Cancer Inst* 2005;97:147–50.
- (2) Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. HPV genotype distribution in low-grade cervical lesions: comparison by geographical region with cervical cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1157–64.
- (3) Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Br J Cancer* 2003;89:101–5.
- (4) Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003;88:63–73.
- (5) Wright TC Jr, Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol* 2004;103:304–9.
- (6) Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27.

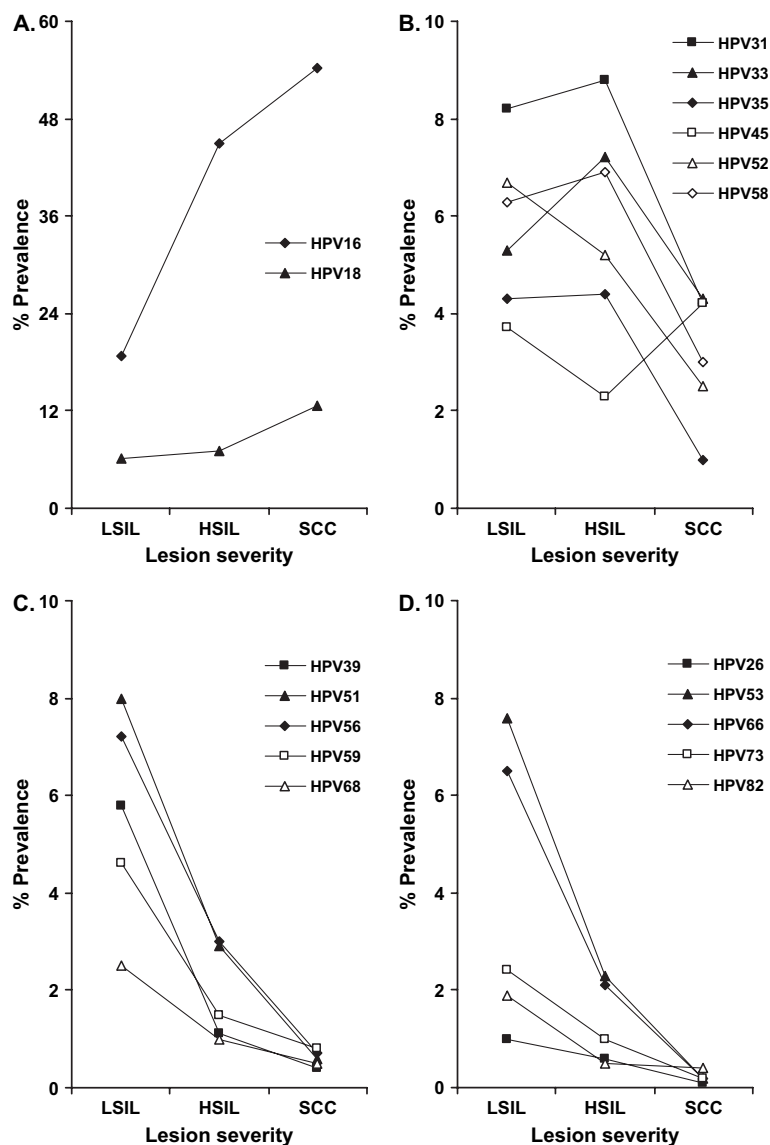
- (7) Naucler P, Da Costa FM, Ljungberg O, Bugalho A, Dillner J. Human papillomavirus genotypes in cervical cancers in Mozambique. *J Gen Virol* 2004;85:2189–90.

## NOTES

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**Fig. 1.** Prevalence of human papillomavirus (HPV) types in cervical lesions of increasing severity. Prevalence estimates are derived from three published systematic reviews of type-specific HPV prevalence data in i) low-grade squamous intraepithelial lesions (LSILs) (2), ii) high-grade squamous intraepithelial lesions (HSILs) (3), and iii) squamous cell cervical carcinoma (SCC) (4). The 13 HPV types included in the Food and Drug Administration (FDA)-approved HPV test kit are shown in A–C: A) HPV16 and 18, B) HPV types present in 1%–5% of SCC (HPV31, 33, 35, 45, 52, and 58), and C) HPV types present in less than 1% of SCC (HPV39, 51, 56, 59, and 68). The five HPV types not currently included in the FDA-approved test set, as discussed by Schiffman et al. (1) (HPV26, 53, 66, 73, and 82), are shown in D.

## RESPONSE

The use of human papillomavirus (HPV) DNA testing for triage of atypical squamous cells (ASCs or equivocal cytologic specimens) is already widespread, and its promise for general screening is gaining acceptance (1). The value of impending widespread HPV testing will depend on how thoughtfully it is used.

As supported by Franceschi and Clifford's statistically powerful contribution, epidemiologists are reaching

agreement concerning which HPV genotypes should be included in diagnostic kits. There is little residual controversy over which types convey highest, intermediate, low, or virtually no risk of cancer. Also, as concluded recently by an IARC expert panel (2), not all potentially carcinogenic types of HPV automatically merit inclusion in test kits because the slight gains in sensitivity from the marginally oncogenic types are offset by substantial numbers of unnecessary referrals (3). Formal cost-benefit analyses considering societal preferences must guide the choices of which HPV types to target in cancer prevention.

Moving forward, it is worth considering which other HPV diagnostic issues are resolved, which answers are emerging, and which questions remain.

It would clearly be desirable to have additional Food and Drug Administration (FDA)-approved HPV tests. However, new diagnostic assays must be validated using data regarding prediction of risk of cancer and cervical intraepithelial neoplasia grade 3 (CIN3) from large, representative study populations. In addition to targeting the correct genotypes, HPV tests must have clinically validated viral load cut points. The detection of infections at very low viral loads substantially decreases the predictive value of a positive test while providing only a minimal increase in reassurance against cancer risk (4).

Test performance cannot be predicted by laboratory experiments or

small demonstrations (5). For example, without performance data, it would have been impossible to predict that the prototype polymerase chain reaction assay that we analyzed for its ability to triage ASCs (3) would be slightly less sensitive (i.e., detecting fewer CIN3 or cancer) and more specific than the FDA-approved assay (Table 1), not the opposite as would have been expected from laboratory experiments. Any claims about new HPV assays should be based on published or openly available data; in particular, laboratories offering "homebrew" tests must meet this standard for their claims to be regarded as credible given the complexity of development of HPV diagnostics (5).

There is an emerging consensus that the next generation of HPV tests should provide some degree of typing (rather than collective detection of pooled types) to permit monitoring of type-specific HPV persistence, the necessary risk factor for cervical cancer. At a minimum, it will probably be important to distinguish HPV16 and HPV18 from the remaining carcinogenic types to permit more aggressive management commensurate with the higher risk of cancer that these types confer (6).

Important unresolved diagnostics and management issues center first on how to define viral persistence. How long should a woman ( $\geq 30$  years old) with oncogenic HPV infection and normal cytology be followed before referral to colposcopy (7)? If colposcopy shows

no  $\geq$ CIN2, how long should a woman who has a persistent oncogenic HPV infection be followed without treatment?

More generally, we need practical protocols for combining HPV detection, cytology, and colposcopy given that each has clear deficiencies for screening and patient management. These protocols must be adapted to low-resource settings in the United States and internationally, where cervical cancer is a major problem.

Finally, a future challenge is to develop assays that permit better prediction of viral persistence and cancer risk than HPV DNA detection alone. This would revolutionize the already dynamic HPV diagnostic field.

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## REFERENCES

- (1) IARC Handbooks of Cancer Prevention. Cervical Cancer Screening. Lyon (France): IARC Press, 2005.
- (2) Coglian V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. Carcinogenicity of human papillomaviruses. *Lancet Oncol* 2005;6:204.
- (3) Schiffman M, Khan MJ, Solomon D, Herrero R, Wacholder S, Hildesheim A, et al. A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J Natl Cancer Inst* 2005;97:147-150.
- (4) Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. *JAMA* 2000;283:87-93.
- (5) Stoler M. HPV testing in cervical cytology practice: it's all about choice. *Acta Cytol* 2005;49:117-119.
- (6) Castle PE, Solomon D, Schiffman M, Wheeler CM. HPV16 infection and the two-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. *J Natl Cancer Inst*. In press.
- (7) Wright TC Jr., Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. *N Engl J Med* 2003;348:489-490.

## NOTES

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**Table 1.** A comparison of clinical performance of the polymerase chain reaction (PCR) test (4) and the Food and Drug Administration-approved HC2 test for the detection of cervical intraepithelial neoplasia grade 3 (CIN3) or cancer ( $\geq$ CIN3)\*

Test parameter	PCR	HC2	P
True negative for $\geq$ CIN3	1629	1497	
False negative for $\geq$ CIN3	35	21	
False positive for $\geq$ CIN3	1299	1431	
True positive for $\geq$ CIN3	243	257	
Sensitivity	87.4% (82.9% to 91.1%)	<b>92.5%</b> (88.7% to 95.3%)	.002
Specificity	<b>55.6%</b> (53.8% to 57.5%)	51.1% (49.3% to 53.0%)	<.001
Positive predictive value	15.8% (14.0% to 17.7%)	15.2% (13.5% to 17.0%)	NS
Negative predictive value	97.9% (97.1% to 98.5%)	98.6% (97.9% to 99.1%)	NS

\*Clinical parameters were calculated for detection of 2-year cumulative incidence of CIN3 or cancer. McNemar's  $\chi^2$  was used to test for statistical significance of differences in sensitivity and specificity between tests; a modified McNemar's  $\chi^2$  was used to test for statistical differences in predictive values between tests. Bold type indicates which test is statistically superior. NS = not significant.  $\kappa$  value = 0.70 (95% confidence interval = 0.68-0.73); % total agreement = 85%; % positive agreement = 74%. Women missing either test result were excluded; the percentages for PCR are therefore slightly different than those given in Schiffman et al. (3).

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